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Your Reference: 10/089,058
Our Reference: 10447-22

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The Commissioner of Patents
& Trademarks
Washington, D.C. 20231
U.S.A.

Dear Sir:

Re: VOLUNTARY AMENDMENT AND SEQUENCE LISTING
United States Patent Application No. 10/089,058
Entitled: Improved Ricin-Like Toxins For Treatment of Cancer
Inventors: Curtis Braun, Admir Purac and Thor Borgford
International Filing Date: October 4, 2001

The present letter is to file a Voluntary Amendment and amended Sequence Listing to the above referenced patent application.

Please amend the application as follows:

In the Disclosure

Please replace the paragraphs beginning at page 10, line 8 to page 12, line 35, with the following rewritten paragraphs:

--Figure 1A shows the nucleotide sequence of the MMP-9 linker region of pAP301 (SEQ ID NOS:1-4);

Figure 1B shows the nucleotide sequence of the pAP301 insert containing ricin and the MMP-9 linker (SEQ ID NO:5);

Figure 1C shows the amino acid sequence of the PAP301 linker and the wild type ricin linker (SEQ ID NOS:6-7);

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please send your reply to:

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Figure 2A shows the nucleotide sequence of the MMP-9 30 linker region of pAP302 (SEQ ID NOS:8-11);

Figure 2B shows the nucleotide sequence of the pAP302 insert containing ricin and the MMP-9 linker (SEQ ID NO:12);

Figure 2C shows the amino acid sequence of the PAP302 linker and the wild type ricin linker (SEQ ID NOS:13-14);

Figure 3A shows the nucleotide sequence of the MMP-9 linker region of pAP303 (SEQ ID NOS:15-18);

Figure 3B shows the nucleotide sequence of the pAP303 insert containing ricin and the MMP-9 linker (SEQ ID NO:19);

Figure 3C shows the amino acid sequence of the PAP303 linker and the wild type ricin linker (SEQ ID NOS:20-21);

Figure 4A shows the nucleotide sequence of the MMP-9 linker region of pAP304 (SEQ ID NOS:22-25);

Figure 4B shows the nucleotide sequence of the pAP304 insert containing ricin and the MMP-9 linker (SEQ ID NO:26);

Figure 4C shows the amino acid sequence of the PAP304 linker and the wild type ricin linker (SEQ ID NOS:27-28);

Figure 5A shows the nucleotide sequence of the MMP-9 linker region of pAP305 (SEQ ID NOS:29-32);

Figure 5B shows the nucleotide sequence of the pAP305 insert containing ricin and the MMP-9 linker (SEQ ID NO:33);

Figure 5C shows the amino acid sequence of the PAP305 linker and the wild type ricin linker (SEQ ID NOS:34-35);

Figure 6A shows the nucleotide sequence of the MMP-9 linker region of pAP308 (SEQ ID NOS:36-39);

Figure 6B shows the nucleotide sequence of the pAP308 insert containing ricin and the MMP-9 linker (SEQ ID NO:40);

Figure 6C shows the amino acid sequence of the PAP308 linker and the wild type ricin linker (SEQ ID NOS:41-42);

Figure 7A shows the nucleotide sequence of the MMP-9 linker region of pAP309 (SEQ ID NOS:43-46);

Figure 7B shows the nucleotide sequence of the pAP309 insert containing ricin and the MMP-9 linker (SEQ ID NO:47);

Figure 7C shows the amino acid sequence of the PAP309 linker and the wild type ricin linker (SEQ ID NOS:48-49);

Figure 8A shows the nucleotide sequence of the UPA linker region of pAP313 (SEQ ID NOS:50-53);

Figure 8B shows the nucleotide sequence of the pAP313 insert containing ricin and the UPA linker (SEQ ID NO:54);

Figure 8C shows the amino acid sequence of the PAP313 linker and the wild type ricin linker (SEQ ID NOS:55-56);

Figure 9A shows the nucleotide sequence of the UPA linker region of pAP314 (SEQ ID NOS:57-60);

Figure 9B shows the nucleotide sequence of the pAP314 insert containing ricin and the UPA linker (SEQ ID NO:61);

Figure 9C shows the amino acid sequence of the PAP314 linker and the wild type ricin linker (SEQ ID NOS:62-63);

Figure 10A shows the nucleotide sequence of the UPA linker region of pAP315 (SEQ ID NOS:64-67);

Figure 10B shows the nucleotide sequence of the pAP315 insert containing ricin and the UPA linker (SEQ ID NO:68);

Figure 10C shows the amino acid sequence of the PAP315 linker and the wild type ricin linker (SEQ ID NOS:69-70);

Figure 11A shows the nucleotide sequence of the MMP-9 linker region of pAP316 (SEQ ID NOS:71-74);

Figure 11B shows the nucleotide sequence of the pAP316 insert containing ricin and the MMP-9 linker (SEQ ID NO:75);

Figure 11C shows the amino acid sequence of the PAP316 linker and the wild type ricin linker (SEQ ID NOS:76-77);

Figure 12A shows the nucleotide sequence of the MMP-9 linker region of pAP318 (SEQ ID NOS:78-81);

Figure 12B shows the nucleotide sequence of the pAP318 insert containing ricin and the MMP-9 linker (SEQ ID NO:82);

Figure 12C shows the amino acid sequence of the PAP318 linker and the wild type ricin linker (SEQ ID NOS:83-84);

Figure 13A shows the nucleotide sequence of the UPA linker region of pAP320 (SEQ ID NOS:85-88);

Figure 13B shows the nucleotide sequence of the pAP320 insert containing ricin and the UPA linker (SEQ ID NO:89);

Figure 13C shows the amino acid sequence of the PAP320 linker and the wild type ricin linker (SEQ ID NOS:90-91);

Figure 14A shows the nucleotide sequence of the UPA linker region of pAP321 (SEQ ID NOS:92-95);

Figure 14B shows the nucleotide sequence of the pAP321 insert containing ricin and the UPA linker (SEQ ID NO:96);

Figure 14C shows the amino acid sequence of the PAP321 linker and the wild type ricin linker (SEQ ID NOS:97-98);

Figure 15A shows the nucleotide sequence of the UPA linker region of pAP322 (SEQ ID NOS:99-102);

Figure 15B shows the nucleotide sequence of the pAP322 insert containing ricin and the UPA linker (SEQ ID NO:103);

Figure 15C shows the amino acid sequence of the PAP322 linker and the wild type ricin linker (SEQ ID NOS:104-105);

Figure 16A shows the nucleotide sequence of the MMP-9 linker region of pAP323 (SEQ ID NOS:106-109);

Figure 16B shows the nucleotide sequence of the pAP323 insert containing ricin and the MMP-9 linker (SEQ ID NO:110);

Figure 16C shows the amino acid sequence of the PAP323 linker and the wild type ricin linker (SEQ ID NOS:111-112);

Figure 17A shows the nucleotide sequence of the MMP-9 linker region of pAP324 (SEQ ID NOS:113-116);

Figure 17B shows the nucleotide sequence of the pAP324 insert containing ricin and the MMP-9 linker (SEQ ID NO:117);

Figure 17C shows the amino acid sequence of the PAP324 linker and the wild type ricin linker (SEQ ID NOS:118-119);

Figure 18A shows the nucleotide sequence of the MMP-9 linker region of pAP325 (SEQ ID NOS:120-123);

Figure 18B shows the nucleotide sequence of the pAP325 insert containing ricin and the MMP-9 linker (SEQ ID NO:124);

Figure 18C shows the amino acid sequence of the PAP325 linker and the wild type ricin linker (SEQ ID NOS:125-126);--

Please replace the paragraph beginning at page 29, line 7 with the following rewritten paragraph:

--Oligonucleotides, corresponding to the extreme 5' and 3' ends of the preproricin gene were synthesized and used to PCR amplify the gene. Using the cDNA sequence for preproricin (Lamb et al., Eur. J. Biochem., 145:266-270, 1985), several oligonucleotide primers were designed to flank the start and stop codons of the preproricin open reading frame. The oligonucleotides were synthesized using an Applied Biosystems Model 392 DNA/RNA Synthesizer. First strand cDNA synthesis was primed using the oligonucleotide Ricin1729C. Three micrograms of total RNA was used as a template for oligo Ricin1729C (5'-ATAACTTGCTGCTCCTTCA-3') (SEQ ID NO:127) primed synthesis of cDNA using Superscript II Reverse Transcriptase (BRL) following the manufacturer's protocol.--

Please replace the paragraph beginning at page 29, line 18 with the following rewritten paragraph:

--The first strand cDNA synthesis reaction was used as template for DNA amplification by the polymerase chain reaction (PCR). The preproricin cDNA was amplified using the upstream primer Ricin-99 (5'-CCGGGAGGAAATCTATTGTAAT-3') (SEQ ID NO:128) and the downstream primer Ricin1729C with Vent DNA polymerase (New England Biolabs) using standard procedures (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor Laboratory Press, 1989)). Amplification was carried out in a Biometra thermal cycler (TRIO-Thermalcycler) using the following cycling parameters: denaturation 95°C for 1 min., annealing 52°C for 1 min., and extension 72°C for 2 min., (33 cycles), followed by a final extension cycle at 72°C for 10 min. The 1846 bp amplified product was fractionated on an agarose gel (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor Laboratory Press, 1989)), and the DNA purified from the gel slice using Qiaex resin (Qiagen) following the manufacturer's protocol. The purified PCR fragment encoding the preproricin cDNA was then ligated (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor Laboratory Press, 1989)) into an Eco RV digested pBluescript II SK plasmid (Stratagene), and used to transform competent XL1-Blue cells (Stratagene). Positive clones were confirmed by restriction digestion of purified plasmid DNA. Plasmid DNA was extracted using a Qiaprep Spin Plasmid Miniprep Kit (Qiagen).--

Please replace the paragraph beginning at page 30, line 5 with the following rewritten paragraph:

--pAP144 cut with EcoRI was used as target for PCR pairs employing the Ricin109-Eco oligonucleotide (Ricin-109Eco primer: 5'-GGAGGAATCCGGAGATGAAACCGGGAGGAAATACTATTGTAAT-3) (SEQ ID NO:129) and a mutagenic primer for the 5' half of the linker as well as the Ricin1729PstI primer (Ricin 1729-PstI: 5'-GTAGGCGCTGCAGATAACTTGCTGTCCTTCAG-3) (SEQ ID NO:130) and a mutagenic primer for the 3' half of the linker. The cycling conditions used for the PCRs were 98 degrees C for 2 min.; 98°C 1 min., 52°C 1 min., 72°C 1 min. 15 sec. (30 cycles); 72 degrees C 10 min.; 4 degrees C soak. The PCR products were then digested by EcoRI and PstI respectively, electrophoresed on an agarose gel, and the bands purified by via glass wool spin columns. Triple ligations comprising the PCR product pairs (corresponding halves of the new linker) and pVL1393 vector digested with EcoRI and PstI were carried out. Recombinant clones were identified by restriction digests of plasmid miniprep DNA and the altered linkers confirmed by DNA sequencing. Note that all altered linker variants were cloned directly into the pVL1393 vector.--

Please insert Sequence Listing pages 45-103 enclosed herewith into the specification.

In the Claims:

Please renumber claim pages 45-51 as claim pages 104-110.

Please amend claims 9, 10, 21, 41 and 42 as follows:

9. (Twice Amended) A nucleic acid molecule according to claim 1 having a nucleic acid sequence selected from the group consisting of the nucleic acid sequence of pAP301 as shown in Figure 1B (SEQ ID NO:5); the nucleic acid sequence of pAP302 as shown in Figure 2B (SEQ ID NO:12); the nucleic acid sequence of pAP303 as shown in Figure 3B (SEQ ID NO:19); the nucleic acid sequence of pAP304 as shown in Figure 4B (SEQ ID NO:26); the nucleic acid sequence of pAP305 as shown in Figure 5B (SEQ ID NO:33); the nucleic acid sequence of pAP308 as shown in Figure 6B (SEQ ID NO:40); the nucleic acid sequence of pAP309 as shown in Figure 7B (SEQ ID NO:47); the nucleic acid sequence of pAP313 as shown in Figure 8B (SEQ ID NO:54); the nucleic acid sequence of pAP314 as shown in Figure 9B (SEQ ID NO:61); the nucleic acid sequence of pAP315 as shown in Figure 10B (SEQ ID NO:68); the nucleic acid sequence of pAP316 as shown in Figure 11B (SEQ ID NO:75); the nucleic acid sequence of pAP318 as shown in Figure 12B (SEQ ID NO:82); the nucleic acid sequence of pAP320 as shown in Figure 13B (SEQ ID NO:89); the nucleic acid sequence of pAP321 as shown in Figure 14B (SEQ ID NO:96); the nucleic acid sequence of pAP322 as shown in Figure 15B (SEQ ID NO:103); the nucleic acid sequence of pAP323 as shown in Figure 16B (SEQ ID NO:110); the nucleic acid sequence of pAP324 as shown in Figure 17B (SEQ ID NO:117); and the nucleic acid sequence of pAP325 as shown in Figure 18B (SEQ ID NO:124).

10. (Twice Amended) A nucleic acid molecule according to claim 1 wherein the nucleotide sequence of the linker is selected from the group consisting of: the nucleic acid sequence of pAP301 as shown in Figure 1A (SEQ ID NO:4); the nucleic acid sequence of pAP302 as shown in Figure 2A (SEQ ID NO:11); the nucleic acid sequence of pAP303 as shown in Figure 3A (SEQ ID NO:18); the nucleic acid sequence of pAP304 as shown in Figure 4A (SEQ ID NO:25); the nucleic acid sequence of pAP305 as shown in Figure 5A (SEQ ID NO:32); the nucleic acid sequence of pAP308 as shown in Figure 6A (SEQ ID NO:39); the nucleic acid sequence of pAP309 as shown in Figure 7A (SEQ ID NO:46); the nucleic acid sequence of pAP313 as shown in Figure 8A (SEQ ID NO:53); the nucleic acid sequence of pAP314 as shown in Figure 9A (SEQ ID NO:60); the nucleic acid sequence of pAP315 as shown in Figure 10A (SEQ ID NO:67); the nucleic acid sequence of pAP316 as shown in Figure 11A (SEQ ID NO:74); the nucleic acid sequence of pAP318 as shown in Figure 12A (SEQ ID NO:81); the nucleic acid sequence of pAP320 as shown in Figure 13A (SEQ ID NO:88); the nucleic acid sequence of pAP321 as shown in Figure 14A (SEQ ID NO:95); the nucleic acid sequence of pAP322 as shown in Figure 15A (SEQ ID NO:102); the nucleic acid sequence of pAP323 as shown in Figure 16A (SEQ ID NO:109); the nucleic acid sequence of pAP324 as shown in Figure 17A (SEQ ID NO:116); and the nucleic acid sequence of pAP325 as shown in Figure 18A (SEQ ID NO:123).

21. (Amended) A recombinant protein of claim 13 wherein the linker amino acid sequence is selected from the group consisting of: the amino acid sequence of PAP301 as shown in Figure 1C (SEQ ID NO:7); the amino acid sequence of PAP302 as shown in Figure 2C (SEQ ID NO:14); the amino acid sequence of PAP303 as shown in Figure 3C (SEQ ID NO:21); the amino acid sequence of PAP304 as shown in Figure 4C (SEQ ID NO:28); the amino acid sequence of PAP305 as shown in Figure 5C (SEQ ID NO:35); the amino acid sequence of PAP308 as shown in Figure 6C (SEQ ID NO:42); the amino acid sequence of PAP309 as shown in Figure 7C (SEQ ID NO:49); the amino acid sequence of PAP313 as shown in Figure 8C (SEQ ID NO:56); the amino acid sequence of PAP314 as shown in Figure 9C (SEQ ID NO:63); the amino acid sequence of PAP315 as shown in Figure 10C (SEQ ID NO:70); the amino acid sequence of PAP316 as shown in Figure 11C (SEQ ID NO:77); the amino acid sequence of PAP318 as shown in Figure 12C (SEQ ID NO:84); the amino acid sequence of PAP320 as shown in Figure 13C (SEQ ID NO:91); the amino acid sequence of PAP321 as shown in Figure 14C (SEQ ID NO:98); the amino acid sequence of PAP322 as shown in Figure 15C (SEQ ID NO:105); the amino acid sequence of PAP323 as shown in Figure 16C (SEQ ID NO:112); the amino acid sequence of PAP324 as shown in Figure 17C (SEQ ID NO:119); and the amino acid sequence of PAP325 as shown in Figure 18C (SEQ ID NO:126).

41. (Amended) A purified and isolated nucleic acid molecule having a nucleic acid sequence selected from the group consisting of: the nucleic acid sequence of pAP301 as shown in Figure 1A (SEQ ID NO:4); the nucleic acid sequence of pAP302 as shown in Figure 2A (SEQ ID NO:11); the nucleic acid sequence of pAP303 as shown in Figure 3A (SEQ ID NO:18); the nucleic acid sequence of pAP304 as shown in Figure 4A (SEQ ID NO:25); the nucleic acid sequence of

pAP305 as shown in Figure 5A (SEQ ID NO:32); the nucleic acid sequence of pAP308 as shown in Figure 6A (SEQ ID NO:39); the nucleic acid sequence of pAP309 as shown in Figure 7A (SEQ ID NO:46); the nucleic acid sequence of pAP313 as shown in Figure 8A (SEQ ID NO:53); the nucleic acid sequence of pAP314 as shown in Figure 9A (SEQ ID NO:60); the nucleic acid sequence of pAP315 as shown in Figure 10A (SEQ ID NO:67); the nucleic acid sequence of pAP316 as shown in Figure 11A (SEQ ID NO:74); the nucleic acid sequence of pAP318 as shown in Figure 12A (SEQ ID NO:81); the nucleic acid sequence of pAP320 as shown in Figure 13A (SEQ ID NO:88); the nucleic acid sequence of pAP321 as shown in Figure 14A (SEQ ID NO:95); the nucleic acid sequence of pAP322 as shown in Figure 15A (SEQ ID NO:102); the nucleic acid sequence of pAP323 as shown in Figure 16A (SEQ ID NO:109); the nucleic acid sequence of pAP324 as shown in Figure 17A (SEQ ID NO:116); and the nucleic acid sequence of pAP325 as shown in Figure 18A (SEQ ID NO:123).

42. (Amended) A linker protein having an amino acid sequence selected from the group consisting of: the amino acid sequence of PAP301 as shown in Figure 1C (SEQ ID NO:7); the amino acid sequence of PAP302 as shown in Figure 2C (SEQ ID NO:14); the amino acid sequence of PAP303 as shown in Figure 3C (SEQ ID NO:21); the amino acid sequence of PAP304 as shown in Figure 4C (SEQ ID NO:28); the amino acid sequence of PAP305 as shown in Figure 5C (SEQ ID NO:35); the amino acid sequence of PAP308 as shown in Figure 6C (SEQ ID NO:42); the amino acid sequence of PAP309 as shown in Figure 7C (SEQ ID NO:49); the amino acid sequence of PAP313 as shown in Figure 8C (SEQ ID NO:56); the amino acid sequence of PAP314 as shown in Figure 9C (SEQ ID NO:63); the amino acid sequence of PAP315 as shown in Figure 10C (SEQ ID NO:70); the amino acid sequence of PAP316 as shown in Figure 11C (SEQ ID NO:77); the amino acid sequence of PAP318 as shown in Figure 12C (SEQ ID NO:84); the amino acid sequence of PAP320 as shown in Figure 13C (SEQ ID NO:91); the amino acid sequence of PAP321 as shown in Figure 14C (SEQ ID NO:98); the amino acid sequence of PAP322 as shown in Figure 15C (SEQ ID NO:105); the amino acid sequence of PAP323 as shown in Figure 16C (SEQ ID NO:112); the amino acid sequence of PAP324 as shown in Figure 17C (SEQ ID NO:119); and the amino acid sequence of PAP325 as shown in Figure 18C (SEQ ID NO:126).

Please add new claims 43-47 as follows:

43. (New) A nucleic acid molecule according to claim 1 having the nucleic acid sequence of pAP304 as shown in Figure 4B (SEQ ID NO:26).

44. (New) A nucleic acid molecule according to claim 1 wherein the linker has the nucleic acid sequence of pAP304 as shown in Figure 4A (SEQ ID NO:25).

45. (New) A recombinant protein of claim 13 wherein the linker sequence has the amino acid sequence of pAP304 as shown in Figure 4C (SEQ ID NO:28).